bio-protocol Preprint

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Fixed tissue preparation, immunohistochemistry, and imaging

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An abbreviated version of this protocol was published in eLIFE in Feb 2015

Corelease of acetylcholine and GABA from cholinergic forebrain neurons

DOI: 10.7554/eLife.06412

Detailed protocol

Immunostaining Protocol for ChAT

Blocking Buffer:
5% Horse Serum in PBS
+ 0.3% Triton-X

For 25 mL:

- *2.5 mL 10 X PBS*
- *1.25 mL Horse Serum*
- *375 µL Triton- X (from 20%Triton-X stock)*
- *20.875 ddH_20*

Antibody labeling solution:

Dilute 1° and 2° antibodies in Blocking buffer as specified below (1:00 1° and 1:500 2° for ChAT, these will change for other antibodies)

Protocol:

1. Add blocking buffer to slices, **shake at RT for 1 hr.**
2. Aspirate Block buffer (*no washing necessary*)
3. Add 400 uL/well of 1° antibody (goat α-ChAT), 1:100 dilution
4. Shake **O/N at 4°**
5. Wash min. 3x in PBS, **5 minutes shaking inbetween washes**
6. Add 400 µL/well of 2°antibody (ex. α -Goat 594), 1:500 dilution
7. Shake at **RT for 2 hr**
8. Wash min. 3x in PBS
9. Mount on slides **with DAPI**

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Granger, A. (2020). Fixed tissue preparation, immunohistochemistry, and imaging. Bio-protocol Preprint. bio-protocol.org/prep656.
2. Saunders, A., Granger, A. J. and Sabatini, B. L.(2015). Corelease of acetylcholine and GABA from cholinergic forebrain neurons. eLIFE. DOI: 10.7554/eLife.06412